BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

JULY 1945

INFLUENZA: METHODS OF STUDY AND CONTROL[1]

The Wesley M. Carpenter Lecture[2]

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THAT influenza should be chosen as the subject for the Wesley M. Carpenter Lecture is a signal distinction and a recognition of the developments in the field during the past decade. In this time we move from the first identification in the Western Hemisphere of virus from epidemics of influenza in man, to the demonstration that prophylaxis against the epidemic disease is possible. In the advance there have been numerous contributors, many diversions and frequent discoveries of fundamental biological importance.

The probabilities for the control of an infectious disease increase as knowledge of its biology advances. There is no simple formula leading to a successful conclusion. Once the responsible agent is identified, however, information develops as tools and methods for promoting the study are devised. But in the study interest in the method should not

[1] From the Virus Laboratory and the Department of Epidemiology, School of Public Health, University of Michigan. Certain of these studies were conducted under the auspices of the Commission on Influenza, Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, U. S. Army.

[2] Given October 13, 1944 at the Seventeenth Graduate Fortnight of The New York Academy of Medicine.

obscure the problem of the disease: its identification, an understanding of the mechanisms by which it is produced, maintained, and distributed; an evaluation of procedures which might aid in the control or cure of the disease.

Since 1933, when Smith, Andrewes and Laidlaw,[1] following on the heels of Shope's[2] demonstration that a virus was involved in swine influenza, first identified a virus from human influenza, a large number of investigators in numerous laboratories in different countries have tackled the various aspects of the problem. While it is impossible to make a sharp division between studies of the virus and the application of this information to study of the natural disease it may be well first to review certain characteristics of the virus which serve as the basis for much of the wider research.

Studies of the Virus

Media: At present there is no direct method for the rapid identification of influenza virus in the clinical case. Its recognition depends upon the demonstration of effects produced in the course of its infectious activity. At first the response of the ferret was the sole indicator; then the mouse, squirrel, hamster and other species came into use. Later the embryonated hen's egg became important, with infection established by inoculation onto the choroallantoic membrane, into the amniotic sac, directly into the embryo, or simply of unfiltered throat washings into the allantoic sac. Beyond a characteristic febrile reaction in the ferret, demonstration of the virus' presence in animals, after intranasal inoculation of suspected material, comes from the development of a typical pulmonary lesion with continued passage or through the fact that the virus induces the production of antibodies in the blood and a state of resistance to reinfection. Originally the egg had somewhat the same general purpose as tissue cultures, for demonstration that virus was present depended upon secondary transfer to animals or serological procedures. In the important discovery first reported by Hirst,[3] later also by McClelland and Hare,[4] that influenza virus caused agglutination of erythrocytes of the embryonic or adult chicken an extremely adaptable reaction came into use. When a certain concentration of virus in the egg is reached it can be readily indicated by an immediate macroscopic effect. The possibility of rapid identification of influenza virus in the patient's secretions has been thereby enhanced so that in at least

two instances the actual nature of an outbreak was established in about 4 days by inoculation of eggs.[5, 6]

Antigenic Variation: Two types of virus are at present identified. Type A is the group of strains resembling the original WS strain of 1933. Strains of swine influenza also have antigenic relationship to Type A virus.[7, 8] For a period it appeared on the basis of procedures employed at the time that the strains were antigenically identical but, together with Magill in 1937,[9] it was shown by more precise methods that even the strains exhibited detectable variations. As these studies expanded, more striking differences among them became apparent.[10] In the eggs the strain differences appear to be accentuated to such an extent that it represents one of the hazards of the method. On the other hand there is the possibility that the antigenic status as determined early in the egg is more truly representative of the human virus as it moves at large than that adapted to animals by repeated passages. Burnet has suggested the significance of still finer differences. He noted with one strain adapted to eggs by the amniotic sac that there was a greater capacity to agglutinate guinea pig cells than chick cells and that by selective adaptation, only agglutinins for guinea pig cells were developed.[11] Under other conditions the agglutinins for chicken cells developed and overgrew the cavean agglutinins. He suggested that the guinea pig agglutinins were those of the original human strain -O- while agglutinins of chicken cells were the result of a derivative -D- developing in the egg—thus a variant of the original. These interpretations do not appear to be supported in the observations of Rickard and his associates or those of our laboratory where virus has been identified after its first inoculation into eggs, by the agglutination of chicken cells to a high titer without significant differences in its effect on guinea pig cells.[12] Although certain of the variations detectable by increasing refinements in technique seem to be of academic interest, evidence continues to accumulate indicating that they are of practical importance as well.

Type B influenza virus was discovered in 1940 but evidence showed that it had been prevalent in the epidemic of early 1936.[13, 14] Its general behavior in animals and eggs is essentially the same as that of Type A. It has, however, been consistently milder in its pathogenicity for animals and relatively few strains of the virus have been isolated. But despite the otherwise close similarities, immunologically Type B virus

is so distinct from Type A as to represent an entirely different agent. The difference is much greater than the variations between strains of Type A so that hyperimmune serum which largely obliterates strain specificity and overlaps the boundaries between Type A and swine viruses fails to show cross reactions between A and B.[15]

The importance of these sharply distinguished entities to an understanding of the disease problem will be clearly seen later in the discussion.

Serological Reactions: In response to exposure to influenza virus, whether by infection or otherwise, antibodies which may be measured in a variety of ways can be demonstrated in the blood stream. The capacity of the virus to infect animals can be neutralized by mixing it with serum of the recovered animal; the neutralizing or protective antibodies are most commonly measured in mice by determining how little serum added to a known amount of virus is required to prevent infection when the mixture is given intranasally. In this manner it was first shown that a large percentage of human individuals after the first few years of life, presumably as a result of natural infection, had antibodies to influenza A[16, 17] and that despite this fact they might well acquire the disease again with a further rise in titer. The response was so clearly correlated with infection by Type A influenza virus and not with other infections that its specificity was established.[18] Infection with Type B virus has no influence on the titer to A, and vice versa.[13, 14] And now the demonstration of an increase in titer between serum taken in the acute phase of illness and that in convalescence is generally diagnostic.

Recently, Hirst's observation[19] that antibodies to influenza virus would prevent the virus from agglutinating chicken erythrocytes has resulted in still more simplified serological procedures, which can be carried out rapidly in a general laboratory.

It is interesting in this respect that here again there is a sharp specificity between Type A and Type B viruses in that the respective immune sera inhibit only the homologous virus from causing agglutination.

It is not desirable in the scope of the present discussion to dwell too long on these measures of the virus activity although they represent largely the underlying methods upon which much of the subsequent information is based. There are other lines of physico-chemical

research which have been increasingly attractive. The size of the virus has been measured under a variety of conditions and except for one brief excursion into much smaller realms its diameter is at present still somewhere between 70 to 100 Mu.[20, 21, 22, 23] Efforts to characterize the virus by electron microscopy have yielded photographs of rounded bodies with relatively high homogenicity which appear to possess the virus activity.[22, 24, 25] The active component freed from much extraneous material has also been obtained in sufficient amounts to foster attempts to identify the chemical composition of the influenza viruses. But at the risk of appearing to minimize the importance of these splendid studies of fundamental biological significance, I should like to turn to the body of investigations which are more concerned with the clinical and epidemiological aspects of the disease.

Pathogenesis

An understanding of the mode of action of influenza virus is important since it serves to orient many activities which might appear at first glance unrelated. Investigation shows that influenza viruses act quite specifically upon the respiratory tract. Except that certain strains have been shown to be adaptable to the nervous tissue in mice[26, 27] and that an hemorrhagic encephalitis, similar to that produced by many viruses, is observed in infected chick embryos,[28] influenza virus in swine, ferrets, mice, chick embryos and man exerts its pathogenic effect on the epithelium of the respiratory system. When virus is administered in relatively large amounts by pararespiratory routes, the disease is not produced whereas a minute amount intranasally sets up the infection. The injury is largely a destruction of the typical ciliated columnar epithelium which lines the larger respiratory divisions and which the virus reaches ordinarily by entry into the lumen. This is the essential tissue to which protection must be furnished in order to prevent disease. When moderate amounts of virus are given intraperitoneally or intravenously to mice, virus can be recovered from the lungs but pulmonary lesions do not develop. This is probably accounted for by the fact that the virus is on the wrong side of the susceptible cell and only when it floods over into the upper respiratory tract will it gain access to the epithelial lining. The pneumonia which develops in experimental animals, while related to the virus injury, is apparently a lesion secondary to epithelial destruction and the subsequent serous exudation.

The effect of bacteria upon the pathologic process in man is not well known since influenza of recent years has not been associated with a high incidence of complications. In several instances, however, observers have discovered simultaneous prevalence of influenza A or B and hemolytic streptococcal infection without too great evidence of symbiotic effect.[29] Shope has clearly shown, however, the exaggeration of injury produced when swine influenza virus and H. influenzae suis are given together.[30] A mild illness is converted into a serious disease. As yet unpublished studies from our laboratory have demonstrated in mice that strains of H. influenzae, which by themselves are harmless intranasally, when given several days after a minimal non-fatal virus infection is induced, can establish themselves, multiply and bring about a lethal result. Moreover, it appears that in some instances the virulence of the bacterial strain is enhanced.

It is apparent, however, that the virus is the major component in the origin of influenza and the disease which has been studied in man has been largely an uncomplicated infection. Consequently, much of the discussion deals with information gained under these conditions in relation to specific cellular injury by the virus.

The Clinical Disease

During the course of an epidemic of influenza there is a surprising degree of uniformity in the clinical picture presented. Nevertheless, studies of the virus infection reveal, as with most diseases, that severity varies from the unrecognized subclinical invasion in a relatively large proportion of the population to the severe fatal disease in which pulmonary involvement is prominent. But that they represent infection by the same virus has been amply demonstrated by the recovery of the virus and by a study of the serological responses.

On the basis of studies in the past decade influenza A tends to elicit a sharp clinical disease with abrupt onset, fever and pronounced constitutional symptoms of three to four days' duration. Even in the milder cases the course tends to follow the same pattern. On the other hand several reports have noted that in comparison influenza B has had a more gradual onset,[31, 32, 33, 34] the disease was less intense and the duration of the fever shorter. Several of the writers describe the onset as a common cold and some observers have noted in children, especially, almost an absence of significant complaints. These impressions are sub-

stantiated to some extent by our experience with a group under close observation. Influenza B occurred in nearly 25 per cent of the population in a large institution as demonstrated by serological means although only 4 clinical cases were recognized.[35] However, the epidemics of influenza B which were studied in 1936 and 1940 revealed a large body of cases with clinical illness quite typical of epidemic influenza.[13, 31] Nigg and her associates reported 4 cases of fatal pneumonia during a limited outbreak of influenza B.[36] In a prevalence a year ago among a group of 100 elderly women, three fatalities occurred out of thirty-one cases. One point noted in the 1936 and subsequent epidemics of influenza B which differed somewhat from that usually seen in influenza A is the not uncommon tendency to nausea and vomiting.

Further evidence of the clinical features has been gained from infection experimentally induced in human subjects by inhalation of virus.[37-41] It is quite striking that while signs of parenchymal involvement have been noted in a proportion of cases, pneumonia has been a rare complication. The incubation period with experimental influenza A has been 24 to 48 hours and the onset abrupt. Chills or chilliness, fever, cough, headache, general aches and prostration of two to three days' duration have been the rule. Nasal discharge has been less frequent. A considerable degree of lassitude follows the decline of fever. With experimental influenza B the incubation period has in general been shorter, not uncommonly twelve to eighteen hours. This brief incubation period is a striking observation and that it represents the effect of active virus is seen by the fact that irradiated material does not produce clinical disturbance. In addition, the course of experimental influenza B has been milder than that of influenza A; fever has usually been of no more than one day's duration and recovery appears to be more rapid. Even under these circumstances nausea and vomiting has been not infrequent.

The illnesses produced by these two viruses offer certain contrasts with the form of upper respiratory infection most commonly associated with atypical pneumonia. It is quite certainly *not* influenza A or B. The onset is usually much more gradual with symptoms of respiratory irritation appearing early. Nasal congestion, cough, hoarseness, substernal soreness tend to develop progressively but the patient does not usually present the same degree of prostration. The leukocyte count is not so uniformly low. While these differences appear to be of minor

nature, they tend to be quite prominent when seen in large groups.

In the individual patient, however, because of the wide variations that may be encountered the difficulty in diagnosis is increased. And since many of the symptoms can be found related to the onset of a great number of diseases, there is still no simple test which permits a prompt diagnosis at a single glance. Efforts which seek to find specific clinical methods for identifying the various etiological entities have revealed the probability that still other types of influenza virus will be discovered, and that unrelated viruses will be found in some of the respiratory infections which at present prevail. The importance of gaining this knowledge cannot be overemphasized since it constitutes the information through which specific preventive or curative measures can be devised.

Epidemiology

Just as identification of influenza virus infection serves to chart the clinical boundaries so it is a valuable procedure in giving information as to the natural history of the diseases, for in this manner the wanderings of the virus can be detected even when they are not suspected through clinical or epidemiological observation. For several years, together with other investigators, it was my opinion that influenza A was almost exclusively an epidemic disease. This was based to a large extent upon the fact that the periods in which the virus had been shown to be present were occupied by epidemics. Moreover, for nearly nine months in 1940 a constant sampling of respiratory infections in the wards of the Third Medical Division, Bellevue Hospital, was made by I. J. Brightman.[42] The first case of influenza A was identified at the end of this period in December when an epidemic began in the city. There was thus little evidence of scattered sporadic cases.

From the winter of 1932-33 when the virus was first isolated until 1940-41, five outbreaks in alternate years had been identified. The cycle skipped a beat in 1942-43 and the epidemic appeared last winter after a three-year interval. But despite the fact that the usual epidemic did not occur during the winter of 1942-43, influenza A was found to be in operation. In Canada, Hare and others observed a sudden, self-limiting, brief flurry in an Army group in April 1943;[33, 43] in England, spotty group infections unrelated to any general incidence were detected during that summer.[44] In Australia scattered cases were detected in the

same months.[34] In our studies serological evidence was obtained of three possible cases in February and March while in May, 3 definite cases, from one of which virus was recovered, suddenly appeared at an army post without any evidence before or after of an epidemic prevalence.[35] It was not until early November that epidemic disease became obvious. These observations covering wide geographic areas at the same time intervals clearly show that influenza does appear in sporadic fashion and it is not an unlikely probability that these episodes represented the stones from which the 1943-44 epidemic was built.

It has been observed that epidemics vary quite definitely in their scope and intensity. For instance, the outbreak of 1936-37, occurring the world over, was moderately severe and distributed generally throughout the population; while that of 1938 to 1939 represented a mild form recognized largely in institutional groups. The same characteristics were observed in other countries that year. Hence, influenza A has been shown to change the pattern of its infection widely: pandemic, epidemic, endemic or sporadic. It is extremely interesting to point out, however, that the form it takes at a given time tends to typify its behavior at widely distant points.

The smouldering scattered distribution recently detected with influenza A, seems to be the more common experience with influenza B. In most accounts of the past three years there has been reference to influenza B scattering through the population without definite indication of an epidemic unless it be in a limited group.[32, 33, 34, 35, 44] It has been repeatedly mentioned that many cases of unidentified illness occurred at the same time as the cases of influenza B. Observations of the distribution of antibodies to influenza B in the population are in accord with this. One series, as yet unpublished, found much lower titers against B than with A but an occasional individual exhibited a high titer suggesting recent infection. Nevertheless, influenza B can cause widespread marked epidemics such as were observed in 1936 and 1940. Hence, the pattern in influenza B is also inconstant.

The above observations tend to indicate that the influenza viruses are in constant circulation. Evidence for human carriers of the viruses is, however, not present. Shope has suggested that swine influenza virus represents the 1918 human strain[30] and he has observed swine infection with more recent strains of Type A virus.[45] Can swine serve as a reservoir for the human disease? Shope has also presented evidence that

swine influenza virus is maintained in a masked form in the lung worms parasitizing swine from which it erupts when provoking influences disturb the equilibrium.[46] This would account for the storage of virus between epidemics and the explosive manner in which many of them arise. The possibility that some similar mechanism serves to preserve the agent in the human body has not been explored. Nevertheless, there is ample evidence that influenza can be transmitted from man to man and at the moment this seems to fit best the facts concerning the spread of the disease.

Efforts Toward Prevention

The foregoing remarks immediately illustrate certain of the problems which enter into prevention of influenza. But it may limit some of the obscurities if we take as a starting point the thesis that the essential requirement of preventive measures is to prevent the virus from reaching the susceptible respiratory epithelium in such a form as to inflict its characteristic injury. Studies over several years have shown that the respiratory secretions contain antibodies to influenza virus, apparently derived from the blood, which may represent the most efficient first line of defense since when present they are readily available to the tissues which they bathe.[47, 48] They are not constantly present, however, nor are they present in large amounts. Nevertheless, it seems that procedures which augment this mechanism may well be effective in procuring protection from the disease. It has been found that in recovery from natural infection the protective capacity of the nasal secretions is enhanced but there is as yet inadequate information as to how long immunity persists. Indications are that it is much longer than is ordinarily said to be the case, probably for a period of years against the same virus. Nevertheless, if infection be induced by a virus so attenuated as to avoid clinical injury it might be applied as needed and build up, through subclinical infection, a relatively durable resistance. Certain reports by Bull and Burnet[49] have yielded results which they considered suggestive but in our studies under a variety of conditions the response to avirulent strains has been too irregular for widespread testing.[50] In one investigation individuals who had developed clinical disease after inhalation of influenza virus, Type B, were subjected to the same procedure with the same virus 4 months later.[39] About one-third again reacted with well marked clinical disease, clearly

indicating that a solid immunity had not persisted for this interval against the amounts of virus employed in the test. The severity of the test is emphasized by the fact that nineteen of twenty-three control individuals came down at the same time. The possibilities along this line have certainly not been exhausted and there are still many reasons for further investigations of immunization by inhalation.

The most widely studied method for attempting to increase immunity has been that of subcutaneous vaccination. It was stated earlier that subcutaneous inoculation of active virus does not produce infection. In animals it can be shown, however, to result in the production of protective antibodies and of resistance. It has been shown also that with proper subcutaneous vaccination the virus-inactivating capacity of the nasal secretions is also enhanced.[51] A number of studies using preparations of virus derived from mouse lung, tissue culture, chick embryo or allantoic fluid have been tested.[38-41, 52-65] In many instances no disease arose to test the result; in others suggestive results have been obtained.

In the winter of 1942-43 under the auspices of the Commission on Influenza, Army Epidemiological Board, 8,000 people in two institutions were included in a vaccination study which employed inactive virus concentrated from allantoic fluid. Studies of the antibody titers before and after vaccination in 419 of the 4,000 vaccinated individuals revealed that over 90 per cent showed a sharp increase in two weeks.[64] Studies in a smaller group, over a longer period of time, indicated that a slight decline had occurred after 4 months, and at the end of one year the distribution of antibody titers remained well above that before vaccination.[6]

No recognizable epidemic occurred during the winter immediately following vaccination. In an effort to gain information as to what benefit had been attained two groups of approximately 100 individuals each were tested for resistance to infection by intranasal inhalation with Type A and Type B viruses, respectively.

The results indicated that vaccination two to four weeks before infection had a pronounced effect. The influence of vaccination against influenza B[41] appeared to persist during a 4 months' interval while against influenza A this was less evident.[40]

During the epidemic of influenza A which occurred in the winter of 1943-44 the distribution of disease in this population might be con-

strued to indicate a persistence of benefit gained from vaccination a year earlier.

On the basis of the experimental studies just detailed an extensive program for the winter of 1943-44 was planned and carried through by members of the Commission on Influenza in an effort to evaluate the effectiveness of vaccination against the natural disease. Results of previous field trials by other investigators suggested that vaccination had a beneficial effect, although the degree of reduction in incidence of disease in vaccinated as compared with control individuals was not sufficiently great to warrant, without further study, the use of this procedure on a wide scale.

The study of last winter, representing the coördinated efforts of members of the Influenza Commission and their associates in six laboratories in different parts of the country, was conducted in A.S.T.P. units at eight colleges and universities and in five New York medical and dental schools. Approximately 12,500 men were involved.[29]

In New York City two studies were organized, one from the laboratories of the International Health Division of the Rockefeller Foundation, by George K. Hirst, Major Norman Plummer and William Friedewald working in the A.S.T.P. units at the College of the City of New York, Princeton University and Rutgers University; the other, originating from the laboratories of Cornell Medical College, was carried out at the five New York medical and dental colleges and at Cornell University in Ithaca by Major Norman Plummer, Thomas P. Magill and Wilson G. Smillie. Rickard and his associates carried out a study at the University of Minnesota; Hale at the University of Iowa; Eaton and Meiklejohn at the University of California and a similar program was conducted from our laboratory at the University of Michigan.

The same preparation of vaccine was used by all investigators. Virus for the vaccine was grown in chick embryos and harvested and concentrated from the extra-embryonic fluids. The viruses of influenza A and B were included in the vaccine and were rendered non-infectious by the addition of formalin. Alternate men in each company were given a single, subcutaneous injection of 1 cc. of either the virus vaccine or control material.

In seven of the nine units, the interval between completion of vaccination and onset of the epidemic of influenza A varied from seven to

thirty-one days. In two units, City College of New York and the University of Iowa, vaccination was begun after onset of the outbreak.

The incidence of clinical influenza in the vaccinated group comprising 6,263 men was 2.2 per cent and in the control group of 6,211 the incidence was 7.1 per cent. In almost all localities the trend was the same. Marked deviation from the average result was observed in only one unit where little difference between vaccinated and controls was evident. In the majority of units three to four times as many cases occurred in the controls as in the vaccinated groups. In two units, ratios of five and six to one were recorded. The factors responsible for these variations are not yet understood but may become apparent after analyses of the results are completed.

From the combined results of the study in all units, it would appear that vaccination was effective in reducing the incidence of influenza to about one-fourth, assuming that the incidence of 7.1 per cent observed in the control group was the expected incidence in a normal population. That this assumption may not be justifiable is suggested by observations made in totally unvaccinated companies at the University of Michigan where the incidence of influenza was about 20 per cent to 30 per cent as opposed to 8 per cent to 9 per cent in the control half of the vaccination study groups. If these differences are significant, it suggests that vaccination was of benefit to a proportion of the controls by virtue of their dilution with an equal number of vaccinated men with whom they were in constant association. The effect of vaccination in reducing the incidence of influenza may be greater than is indicated by comparing vaccinated and control subjects in a single homogeneous population. This may be another example of a fundamental epidemiological principle that has found application in the control of diphtheria and other epidemic diseases.

At City College of New York and the University of Iowa, where vaccination was begun while the epidemic was in progress, the morbidity rates were the same in treated and untreated groups, until the end of the first week after vaccination. Thereafter the difference became evident.

At the University of Michigan, during the prevalence of influenza A, an attempt was made to determine the effect of vaccination upon the incidence of the milder forms of respiratory illnesses unaccompanied by fever. All cases with symptoms of respiratory disease reporting to

sick call were studied. Those with temperatures of 100° or more were hospitalized while those who exhibited no significant febrile reactions were seen almost daily in the dispensary until symptoms subsided. The great majority of hospitalized cases was diagnosed as influenza. That a high percentage of these was due to the virus of influenza A was confirmed serologically in about 90 per cent of the unvaccinated group. The afebrile illnesses studied in the dispensary were classified as influenza, local respiratory infection, or cold, depending upon the clinical impression. Except for the absence of significant fever, patients with influenza seen in the dispensary presented essentially the same syndrome as did the hospitalized cases. That the virus of influenza A was etiologically related to the illnesses observed in the majority was demonstrated by appropriate laboratory tests. In the group of cases diagnosed as "local respiratory infections," symptoms were confined to the respiratory tract and there were signs suggesting localized infection, such as sinusitis, pharyngitis, etc. The colds consisted of cases in which the presenting and predominant symptoms were those of rhinitis.

A division between vaccinated and controls within each diagnostic category reveals interesting variations. Of the hospitalized influenzas, the greater proportion were contributed by unvaccinated persons, while of the milder cases, such as ordinarily would not come to the attention of physicians in civilian practice, the differential was less marked. Thus, while the incidence of influenza of all degrees of severity was less in the vaccinated half of the population, in a significant proportion of those in whom infection did occur vaccination appears to have reduced the severity of illness.

The fact that no significant difference is apparent in the incidence of cases diagnosed as local respiratory infections and colds in vaccinated and controls, suggests that the vaccine had a rather specific effect. However, serological study indicates that, during the height of the outbreak, a number of these cases probably were mild infections caused by the influenza virus. It would appear that control and vaccinated individuals contributed equally to the incidence of respiratory illness with the mildest manifestations.

A point of interest noted in all study groups was that the difference in incidence of influenza in treated and control groups was greatest during the height of the outbreak, and as the epidemic subsided the difference between the two became less marked.

Results of this set of studies represent for the first time a clearcut demonstration that vaccination with inactivated virus by the subcutaneous route is capable of protecting to a significant degree against natural epidemics of influenza. They do not indicate, however, that the solution is complete. There is still need for the evaluation of the most effective strains to be employed so as to give the widest range of immunity; there is the question of the optimal amount of virus which can be used; beyond a certain point the possibility of toxic reactions enters; how long does the immunity last and can it be bolstered by multiple rather than single doses; what methods of production of material or even of immunization are to be the most practicable? But above all the problems of technique, it must be kept in mind that it is the production of immunity, not the production of virus, with which we are ultimately concerned. It is also important to bear in mind that the presence of antibodies and immunity are not synonymous, especially in the human individual where a ceiling on antibodies seems to exist.

Passive Immunity: The prophylactic approaches which have been discussed are those which tend to induce active immunity by modified infection with active virus or vaccination with inactive material. In both these instances it has been suggested that the effect obtained may reside largely in the influence upon secretions of the respiratory tract. If the superficial cells lining the air passages represent the vulnerable tissues, might it not be possible to protect them by applying immune substances directly at the surface? In other words, the action of the secretions might be augmented by antibodies introduced directly by the respiratory route. Smorodintsev and his associates[66] were the first to report efforts to study this possibility in the human subject. They subsequently stated that by spraying relatively small amounts of serum which were inhaled by the exposed subject, a marked reduction in the incidence of the disease during an epidemic was obtained.

Studies in experimental animals, largely mice, have repeatedly indicated that serum given intranasally has far greater efficacy than when given by other routes. In spraying for prophylactic purposes, however, it should be noted that excessive amounts of serum over long periods of time have been required to permit any significant results to be observed. On this basis alone one might have certain reservations as to the applicability to the problem of human influenza. In an effort to gain an impression of its usefulness, studies among human volunteers were car-

ried out by members of the Influenza Commission. Serum was tested for its capacity to prevent infection by virus sprayed into the nostrils. Details of these studies have not yet been reported but it can be mentioned that substantiation of the statement of the Russian workers was not indicated.

This lecture has touched rather broadly on a wide variety of methods of study which have been employed during the past ten years in the investigations of influenza. It has been seen that knowledge of the viruses, the diseases they produce, and their distribution in nature, have been approached directly. It has become increasingly apparent as well that in order to determine the value of procedures which might serve in the prevention and control of the human disease information must be obtained in human subjects. In this respect somewhat venturesome utilization of the human volunteer has been most profitable, since it permits evaluation of the different proposals in the host concerned. Whether one can consider the experimentally induced infection strictly comparable to the natural disease is not a question, but it does furnish a procedure for investigation. At the moment, the procedure which has given the most definite evidence of limiting susceptibility to influenza has been subcutaneous vaccination. We have indicated, however, that much remains to be done.

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CONCEPTS OF THE IMMUNOLOGY OF CERTAIN VIRUS INFECTIONS*

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THE concepts which we shall discuss represent the combined efforts of several workers who have been with one of us (P. K. O.) in his laboratory over a period of many years. This work, although carried out in collaboration with different associates** at different times, has a thread of continuity which makes it possible to review it as a whole.

In the early days of virus research, not so many years ago, the facts of immunity were held either as strange or mysterious, or as unaccountable. In the light of investigation the air of mystery cleared and it is now known that serological and immunological processes in virus infections are a counterpart of those characterizing microbic or biological agents. Serum antibody such as agglutinin, precipitin and complement-fixing and neutralizing, can be elicited: the statement that virus infections are followed by a lasting immunity to reinfection has many exceptions—repeated or second attacks of common cold, herpes, influenza, foot-and-mouth disease, phlebotomus fever, poliomyelitis and other virus diseases are recorded—and the dictum that only active virus can induce practical active immunity has had no general application. The serological and immunological phenomena were simply obscured by the nature of the infective agent—its size; non-cultivability *in vitro;* its action as an obligate intracellular parasite, or other factors; but once proper techniques were used, the hidden facts became revealed. At the present time one is not so much concerned with the nature of virus immunology as distinct from that of other biological agents, but rather

* Part of the paper read before the Stated Meeting of The New York Academy of Medicine, December 7, 1944.

** Their names are in the References, at the end of the paper.

with the mechanisms underlying immunity and resistance, or more properly, with those of a host's susceptibility to infection.

The general field in which investigations were carried out here is in that of (1) variations in individual host susceptibility which may reflect genetic, maturation or other physiological factors–the so-called "natural resistance"–and (2) individual variations in hosts of uniform primary susceptibility to a virus as studied in experimental animals, the variations being developed either as a result of prior exposure to the virus or of nonspecific factors.* The particular problems included the influence of age on susceptibility to infection; the so-called persistence immunity; the correlation between neutralizing antibody and immunity; certain nonspecific factors of resistance; the nature of the virus neutralization test; comparative immunization by means of active and of inactivated viruses, and passive immunization, including its use in therapy.

One is immediately impressed with the significance and importance of the knowledge of the pathogenesis of an infection for a profitable study of the principles of immunological phenomena. Thus of a malady under investigation, whether naturally occurring or experimentally induced, one should know as far as it is possible, the route by which the virus enters the body; whence it progresses from this point; the tissues in which it then resides or multiplies; the lesions it produces and, after the illness, the way by which the incitant leaves the body, or possibly, how and where it persists. For every topic on immunology in each of the following sections here discussed, the importance of pathogenesis is stressed.

The first of the subjects is on the variations in individual host susceptibility which are brought about with age.

The Influence of Increasing Age on Susceptibility and on the Immune Response

That age of an animal is a factor in determining the degree of immune response to bacterial and protein antigens has long been known. In this laboratory interest was aroused in the problem when it was observed that only infant, but not adult mice succumbed to intraperitoneal injection of vesicular stomatitis virus[1] and again, by the fact that

* Susceptibility or insusceptibility is used in a relative, not absolute, sense for the same host can be susceptible to a virus given in the brain but fails to respond when the virus is introduced into the skin. The degree to which resistance or immunity is lowered would determine susceptibility, and the degree to which either is raised, insusceptibility.

young animals of other species also could be attacked by this and by various other neurotropic viruses given by different peripheral routes.[2]

Whatever may be the mechanism underlying the variations in host susceptibility which develops with age, it is of considerable interest. For it is in part the causal factor in producing the differing types of infection, from the inapparent or latent (or perhaps no infection at all on exposure) to fulminating lethal disease. For example, the age factor may be important epidemiologically in influencing the epidemic distribution in certain age groups in a population as occurs in poliomyelitis.

In this laboratory studies were carried out on a development, *pari passu* with increasing age, a) of pre-existing "barriers" in nervous (and non-nervous) tissues, and b) of an enhanced immune response (serum neutralizing antibody plus immunity to a challenge dose of virus).

Barriers: The assumption here is that host insusceptibility depends on the changes, not as yet completely defined, developing with increasing age in specific tissues which lie in the path of progression of a specific neurotropic virus. In such tissue virus does not multiply and its progression through and beyond that tissue is thus hindered.

To repeat, knowledge of the precise pathogenesis of the disease induced by the virus under study is essential since the barriers as defined are anatomical. Thus it happens that after peripheral inoculation of young mice, the pathway of the stomatitis virus (determined by histology and by subinoculation of tissue) is in a closed system—axonal, neuronal and trans-synaptic—from the peripheral portal to the central nervous system, attack on the latter leading as a rule to fatal encephalitis. In old mice the virus progresses from the point of entry only to a certain site, the barrier, beyond which the animal is apparently unaffected; it therefore survives without showing signs of disease. After intracerebral inoculation of the virus in young or old mice, however, the virus is placed on the other side of the barrier, into susceptible tissue; then it progresses widely and at random throughout the CNS.

In this way the pathogenesis of the disease and the spread of virus from periphery to CNS for different species of animals and of viruses were determined[3-8] and the sites of the barriers mapped. The latter in resistant, older mice were located: after intraocular exposure, in the retina; after intramuscular, the muscle or myoneural junction; and after intranasal instillation, the anterior olfactory area of the brain. In old guinea pigs after intramuscular or pad inoculation, the site was found

at the myoneural junction or specialized nerve endings and after intranasal instillation, between the nasal mucosa and the olfactory bulbs. For Eastern and Western equine viruses in resistant older mice and guinea pigs the barrier was located in the blood vessels.[4-8]

What are the grounds for the concept that adult tissue—the barrier—does not support multiplication of virus, rather than that the virus is stopped in its pathway owing to a generalized immune response? It was shown that vesicular stomatitis virus injected intramuscularly in young mice multiplies actively in the muscles; in old ones no such local multiplication occurs and even though virus persists there it invades neither the sciatic nerve nor the CNS—the pathway of the virus in the young.[3] It is of interest, however, that although old mice are insusceptible to intramuscular inoculation they are still susceptible when the muscle barrier is surmounted by introducing virus into the sciatic nerve whence it progresses to the CNS bringing about fatal ascending myelitis and encephalitis. Again, lack of any appreciable quantity of circulating virus in intraperitoneally injected adults[9] (when viremia is the rule in the young) may be linked with the insusceptibility of peritoneum, since the adult CNS is fully susceptible to infection. Moreover, young, actively multiplying (metabolizing?) tissue is generally more responsive to many viruses and also to tumors (Rivers; Friedewald and Kidd). Also, chick embryos are readily infected by many viruses in contrast to chicks after hatching which become insusceptible to the same incitants.[1] One can conclude, therefore, that with age non-nervous tissue—the "peripheral organism"—becomes increasingly resistant to virus.

Immune Response: Seen from another view[9] age is a factor in determining in the experimental animal its immune response (production of neutralizing antibody plus immunity to a challenge dose of virus). Even though young and old are equally susceptible to equine encephalomyelitis virus introduced into nervous tissue,[10] the response of infant mice to immunization with active or with inactivated virus is slow and feeble; of older ones, rapid and strong, when the challenge dose of virus is introduced extraneurally. The difference in response may account therefore for the increasing insusceptibility to infection developing with age.

The Correlation of Antibody with Immunity to Reinfection

In an elaboration on the role of neutralizing antibody in experi-

mental equine virus infection it was found[9] that during active immunization much less antibody is correlated with immunity to peripheral than to intracerebral administration of a challenge dose of virus. The difference in pathogenesis of the infection progressing from these two sites of injection should[4,5,6] be borne in mind. As described, after peripheral inoculation into normal young mice the virus reaches the CNS via the blood; into vaccinated ones, no virus is detected in the blood—it is neutralized before it can get to the susceptible CNS tissue; after intracerebral inoculation, virus is placed in direct contact with susceptible tissue and the antibody in the blood is therefore of no immediate significance. One can therefore picture the susceptible cell in the CNS as being bathed not by the circulating blood but by interstitial fluid; that its protection would depend on the concentration of neutralizing antibody in this fluid or, in other words, on the availability of antibody to the cell.[11-13] The following elucidates this point:

It was shown[11-13] that the neutralizing capacity of serum and of spinal fluid of rabbits vaccinated with equine virus paralleled each other at a ratio of the order of 300:1—just as has been reported for a bacterial antigen by Freund. That is, when serum dilution of about 1:300 or higher neutralizes virus one can then expect the spinal fluid (also blood-free brain tissue) of a rabbit to contain demonstrable antibody and the animal can then survive an intracerebral test inoculation of large doses. When, however, this serum titer is not reached, no antibody is detectable in the spinal fluid and the rabbit succumbs to infection. The results would suggest therefore that the immunity of the CNS is dependent on the concentration of antibody available to that particular tissue (cf. Fox, in yellow fever of mice).

The factor of availability of antibody to particular tissues applies, of course, to other viruses as well. In foot-and-mouth disease[14] large doses of antiserum given subcutaneously fail to arrest vesicle formation at the site of plantar inoculation of virus but a certain amount of serum, depending on its potency, prevents generalization of lesions (Waldmann and Pape; cf. also Francis' work on influenza). In such animals in which a high level of antibody is induced by means of formolized vaccines, even the local lesions can be prevented (Bedson et al.).

In a practical sense the studies on the correlation of antibody to immunity offer an indicator of the possible effectiveness of a vaccine: an adequate level of induced serum antibody is associated with immun-

ity to infection by peripheral route.[12] The goal in vaccination would therefore be production of a proper level of serum antibody.

The Neutralization Test

By means of a neutralization test one can determine the presence either of a virus or its specific antibody depending on which of the materials is designated the unknown. It is usually performed by adding fluid containing suspected antibody to suspensions harboring virus and after varying periods of contact injecting the mixture, properly controlled, by a specified route.

It is an important test for it is used ordinarily to detect the prevalence of a disease, or the geographical dissemination of a virus or as a method of immunological study. It is therefore natural to seek out the variables of the test; to increase its delicacy and its precision and to study its mechanism.

Earlier observers maintained that the correlation, mentioned in the preceding section, between serum neutralizing capacity and the degree of immunity, did not exist.

The neutralization test is ordinarily carried out using undiluted serum plus virus dilution. In certain circumstances it is important to employ serum dilutions plus a constant amount of virus in order to reveal the true picture. By means of the described "dilution" tests[9,11-13,15,16] a correlation can be deduced between the neutralizing capacity of serum antibody and immunity to test doses given either by way of the brain or extraneurally.

It was furthermore demonstrated[17] that a difference exists in the protective capacities of antisera to the equine viruses which depends on the route of injection of mixtures, for example, whether in mice the route is cerebral or peripheral (peritoneal, muscular). The former method shows 100 or even 10,000 times less neutralizing capacity than the latter, "peripheral," procedure with the identical test materials. Studies are now being carried out which point to the availability of complement in the local, extraneural, tissues for completion of neutralization as one of the mechanisms possibly underlying the enhanced peripheral neutralization.[18,19]

The neutralizing capacity of a given antiserum to the Western equine virus varies also according to other conditions of the test, as for example, use of fresh, frozen, stored or heated serum and incubation

of serum-virus mixtures before injection. To conclude from the results of extensive investigations,[18 20] it would seem that complement or a virus-inhibiting factor, plays at least some part in the neutralization of the equine virus by its antiserum, just as either may be the key to the solution of the problem of greater capacity for virus neutralization by certain routes of injection. What particular substance may be involved is still under study and present investigations include connection of virus-inhibiting substance not only with serum-virus relationships but also with immunity in general.*

Active and Passive Immunization

Immunization with Active and Inactivated Viruses: The virus of equine encephalomyelitis, inactivated especially by means of formalin and in sufficient dosage can reproduce the antigenicity of active virus, as shown by the development of neutralizing antibody and by resistance to test inoculation of virus.[15,16] For example, mice vaccinated with formolized virus develop at 2 weeks fully as high a concentration of serum neutralizing antibody as do mice vaccinated with active virus. The concentration falls in both groups during the following 6 months and at the end of that time is increased markedly by a single dose of active or inactivated virus. So also a similar curve can be shown for the immunity developing to test doses of virus introduced into the brain. Moreover, an immunity can be induced in mice and guinea pigs with vaccines in which no infective agent could be demonstrated by tests made as rigid as possible.[22] Elaborate tests were made, for the absence of multiplying virus is a crucial point in the problem of "persistence" immunity to be discussed later. Can a minute amount of active virue (less than one infective unit) residual in a vaccine and escaping detection, immunize an animal? It was found by test that [17,22] such an amount is ineffective; indeed, a relatively large dose of active virus is needed for successful immunization of normal animals.

Another point of practical significance is that vaccines, to be potent for immunization, should contain a high concentration of virus before inactivation.[22] For the equine virus, the potency of vaccines depends on its use in undiluted state; the immunizing power falls even to its complete extinction when it is diluted 1:10 or higher. The lack of

* An interesting development during these studies was that the equine virus antibody was recovered, after electrophoresis, from the gamma globulin and from the albumin of rabbit antiserum.[21]

sufficient quantity of antigen may also be one of the reasons for the difficulty of immunization with poliomyelitis virus.[23]

Still another practical development is in the use of mouse brain as a source of the equine virus vaccine. It was pointed out[24] that it can replace to advantage infected horse brain which was first used by Shahan and Giltner. Intensive study revealed its effectiveness although chick embryo vaccines have in time supplemented other preparations chiefly for practical reasons (expense and ease of preparation). At present mouse brain vaccines have been recommended for preventive use in experimental St. Louis and Japanese B encephalitis and in man in Russian tickborne encephalitis.

Finally, instead of formalin as an inactivating agent for preparation of antigens for immunization or other purposes, ultraviolet radiation can be used.[25] The antigenicity of such virus rapidly diminishes, however, with further irradiation—an effect which stands in the way of its practical use, an objection applying also to certain other viruses.

With respect to immunization with active virus, early investigations (1925-1928) have indicated that for vesicular stomatitis virus[26] and for foot-and-mouth disease virus,[14] injection into the skin in sites other than the pads of the feet of guinea pigs (in which the viruses cause local vesicular lesions) is followed by an absence of any visible local lesion or of any objectively obvious sign of illness. Yet the viruses become generalized through the circulation and induce after a single sufficient dose a high degree of immunity to reinoculation in the pads (v. supra). It was later found by others that this method can be applied in prevention of certain other virus maladies.*

Passive Immunity: Of the more practical studies in this field as carried out here and to be discussed now are a) blocking effect of antiserum on antigenicity of active and inactivated virus; b) use of antiserum in nasal seroprophylaxis and c) experimental serotherapy.

Investigations on the effect of immune serum on the antigenicity of active and inactivated equine virus demonstrated that the antigenicity of both can be blocked and to the same extent by adequate amount of antiserum.[28] A discussion of the rationale will be passed by, but this can be stressed: the failure of vaccines to bring about immunity when

* By constant association with active equine virus over long periods of time serum neutralizing antibody may be produced to an appreciable degree, as occurred in a laboratory worker exposed to the virus for about 6 years.[27] Such probable inapparent infections followed by production of antibody have been shown later to arise not infrequently in areas where the virus is disseminated and its vector abounds (Howitt; Hammon).

they are injected along with, or some short time after antiserum—a procedure used now and again in practice—is based on this blocking effect.

Nasal instillation of antiserum as a preventive of infection was studied with viruses progressing along the nasal pathway, and it was shown[29] that the method was effective to a certain degree against experimental pseudorabies and Eastern equine virus infections. When antiserum was instilled as a single dose intranasally in guinea pigs, they were protected against lethal infection with the viruses given by way of the nose. The protection was evident from the time of serum instillation until at least 5 but not 24 hours later. This type of passive immunization has recently received renewed impetus from the work of Professor Smorodintsev of Moscow and his colleagues on similar successful prevention of influenza in man. The rationale of the preventive effect may lie in the fact that a sufficient amount of neutralizing antibody is made available locally to susceptible tissues or at the portal of virus entry.

Serotherapy of experimental Western equine virus infection in guinea pigs is effective if antiserum is given in single or multiple doses 24-48 hours after virus inoculation (cf. Zichis and Shaughnessy); it is ineffective if treatment is begun after onset of definite signs of encephalitis. It would thus seem, if sufficient antiserum can be administered in such instances as in laboratory infections in which exposure to virus can be ascertained and even prodromal signs noted, that a hopeful outcome may be expected in an otherwise highly fatal infection.[30]

An interesting side effect was observed during the tests on serotherapy. Several serum-treated animals developed encephalitis after unusually prolonged incubation periods (13 to 47 days). The "delayed reactions" arose when serum-antibody had fallen to a low titer but was still demonstrable. The virus probably persisted in the CNS throughout this long period since it could be neutralized by serum-antibody if it progressed from an extraneural portal to the CNS. The delayed reaction, which further limits the use of serotherapy, occurs therefore when a low serum antibody titer is reached, when antibody content of the CNS becomes ineffective to prevent virus from passing to and infecting other cells.[30]

"Persistence Immunity"

An exciting hypothesis to account for the enduring immunity which follows certain virus infections is that it is correlated with the per-

sistence of virus in the host tissues, commonly called "persistence immunity." If the correlation is to stand, the negative must be true: the host becomes susceptible to infection once the virus leaves him. The proposition, stated perhaps for the first time in 1887 by L. Pfeiffer, who suggested chiefly on the basis of clinical observation that a man is immune to smallpox as long as he carries the virus, has been the subject for study in this laboratory for many years.

Among the many reasons offered for accepting the hypothesis are several cogent ones: (1) Virus neutralizing antibody has been shown to persist for a life time in certain infections characterized by lasting immunity. Sawyer states that serum antibody was found 75 or 78 years after an attack of yellow fever. Since antibody is produced as a reaction to specific antigen (virus) it is assumed that the latter must be present all that time to offer the stimulus although Sawyer declares that with time the antibody may in exceptional instances become undetectable and it is unknown whether "immunity could ever fall so far as to permit a second attack of yellow fever." In other words, tissue immunity to reinfection may be a more sensitive test of the immune state than is circulating, neutralizing antibody.[9,31] (2) For those infections followed by a temporary immunity, the proponents of the persistence hypothesis postulate that the virus attacks, and may persist in susceptible tissue that is superficial and is thus shed, along with the contained virus, within a short time—as possibly in influenza and common colds. (3) Immunization with active virus produces generally durable and solid immunity. (4) In such experiments carried out in immune hosts in whom search for virus failed, it is assumed that the failure offers no evidence that the host is virus free, only that the proper experimental procedure is not or cannot be applied. (5) Virus can persist in immune carriers even though they possess a good amount of neutralizing antibody; it is conjectured that the virus resides within the host's cells. If so this intracellular position affords the virus protection from the antibody (Rous and Jones and others). (6) A significant test with a plant virus as reported by Kunkel is contributory evidence for persistence immunity. The yellows virus can be inactivated at 38-42°C. maintained for 2 weeks, which spares the plant (here the periwinkle). The cured plant can then be re-infected, as if it were normal; it can thus be infected and cured as often as desired.

Are all the correlations as just stated in this relatively simple propo-

sition of enduring immunity being dependent on persistence of virus, purely accidental or fortuitous as some would believe?

In early experiments[32] vaccine virus was recovered from the tissues of an immune rabbit 114 days after inoculation of virus. Later Dresel showed that in similarly tested rabbits virus was recoverable up to but not beyond 8 months after inoculation, although immunity endured at least a year. The subject was studied here anew[33] and it was found that the immune state (i.e. presence of agglutinin and neutralizing antibody plus immunity to reinoculation of virus) was evident 2 or 3 years after infection, at a time when the animals were showing signs of old age. But no virus could then be detected. Vaccinal immunity therefore lasted beyond the persistence of recoverable virus. It should be stressed that the absence may also mean that the quantity of virus is so reduced in the tissues that a single infective unit is difficult to isolate. Moreover, not all tissues were tested, and finally, the virus if masked by antibody would be undetectable by the available methods.

Virus has also been obtained in well and presumably immune animals within about 3-4 weeks of convalescence from experimental infection (foot-and-mouth disease;[14] poliomyelitis in the monkey[34]). These positive results were however exceptional: in foot-and-mouth disease 20 additional cattle examined 20-176 days after experimental infection were negative[14] and in poliomyelitis, additional studies[35] showed that the virus rapidly disappeared (within a few hours) from the tissues of immune animals. Again, in recent tests with the Western equine virus,[36-38] the latter was found at 28 but not at 72 hours in the CNS after its injection into immunized animals.

Furthermore, as opposed to the hypothesis, and placed against item 3 cited above (active virus injection produces solid and durable immunity) are the results of recent experiments[16,22] revealing that animals can be immunized by a sufficient dosage of formalin-inactivated equine virus to at least as high a degree of immunity as that induced by active virus. It is yet to be shown that the immunity following inoculation of active virus can generally equal that following an attack of the disease itself. Practical immunization can therefore be achieved by means of inactivated virus.[12,16,22,25] *

* In this connection, the striking results of Chase should be cited: a chemical antigen (2-4 dinitrochlorobenzene) can induce a satisfactory level of cutaneous hypersensitiveness enduring for at least 7 months after a series of treatment; attempts to recover antigen from the animals failed (personal communication). Sulzberger, who publishes instances of persistence of antibody for several years in persons not exposed again to the antigen, states that "persistence of living microorganisms is not necessary to account for the continued state of cutaneous or other allergy (including the persistence of immunity to reinfection)."

In favor of persistence immunity is the possibility that virus in immune hosts may be masked by neutralizing antibody and therefore is not recoverable by ordinary methods. If this obstacle can be surmounted and if, in addition, the work by Kunkel on plants can be made to apply to mammals, one will be in a better position to affirm the hypothesis. In conclusion, no definite evidence was obtained by the methods thus far used to support the idea of immunity based on persistence of virus.

The Response of the Immune Animal to a Challenge Dose of Virus

Animals (rabbits, guinea pigs, and mice) sufficiently vaccinated with the equine virus exhibit immunity to large test doses of the homologous virus introduced intracerebrally but full susceptibility to the heterologous strain.*

Rabbits or guinea pigs adequately vaccinated to withstand 1000 lethal cerebral doses respond to challenge dose with a characteristic febrile reaction. In vaccinated or nonvaccinated animals the febrile response, a steep rise in temperature first observed 2 hours after inoculation which persists for 24-30 hours, is similar. Thereafter, fever continues in control animals until prostration but in immune ones the temperature drops to normal where it remains.

Since no such characteristic rise in temperature occurs in guinea pigs or rabbits after intracerebral injection of formalin-inactivated virus, or of suspensions of normal mouse cerebral tissue, or of a neutral mixture of virus and antiserum, and since the virus is recovered from the perfused brain of guinea pigs during the febrile period, it is plain that the fever in vaccinated animals reflects virus action and therefore an abortive infection.[37,38]

The confirmation of the phenomenon as an infection was obtained by histological study and by demonstration of fluctuations in the neutralizing antibody in the brain tissue. The cerebral lesions in vaccinated animals were comparable in type and, with few exceptions, in degree with those in nonvaccinated ones, as observed 24-48 hours after virus inoculation. It is noteworthy that vaccinated animals, presumably immune at the time of introduction of the challenge dose do not rid them-

* Two strains of equine encephalomyelitis virus exist in nature, the Eastern and the Western. Both are serologically and immunologically distinct, i.e., there is no cross-neutralization nor cross-immunity in animals recovered from infection or vaccinated with either active or inactivated virus.

selves of the equine virus immediately after exposure, before any damage is done. On the contrary, immune animals permit invasion by the virus, allow its persistence (even though for a short time–here, a day or two), and exhibit during earliest stages definite pathological changes of the same type* and practically of the same degree as those seen in non-immunes. The virus progresses in the latter in its destructive course of necrosis of neurons, of stroma of the CNS, and of vascular walls until the ultimate death of the host. In immunes it acts to gain in the brain only a foothold–actual multiplication of virus cannot be demonstrated–when it meets certain obstacles, the defensive mechanisms, which, as it were, stop it in its tracks. Then it is undetectable, nevertheless, first leaving its impression on the brain in certain lesions which reflect abortive infection. Bedson earlier observed a similar condition in experimental psittacosis: vaccinated, immune mice after test inoculation with virus show no signs of disease, but definite, characteristic lesions in the spleen, in which active virus can be found. The production of lesions–a consequence of the effects of virus–is therefore evidence for an actual infection.

Still other evidence for infection is the variation in "brain" antibody: "serum" antibody ratio. Thus during the febrile period no change occurred in the ratio over that prior to the test virus inoculation. One week after the test dose was given, however, brain antibody rose considerably so that the ratio shifted from about 1:300 to 1:1 or 1:10; the second week after, it returned to about 1:100. How can this be viewed? In an immunized animal, after a test dose is given intracerebrally, an excess of the virus is deposited in a site containing antibody; virus remains in excess for about 24-30 hours; then follows an excess of antibody; in this way the animal overcomes its infection and remains well.[38]

To summarize: the ideal state in immunization by artificial means with active or inactivated virus, that is, one in which the host after any sort of exposure to virus promptly and completely sheds or eliminates any dosage of it is not readily achievable. When an adequate

* Examination of the CNS of stock animals and animals after vaccination revealed no visible changes. Other material such as formolized virus or normal guinea pig brain, inoculated intracerebrally, gave rise to lesions in the same elements as were involved in the earliest stages, within 24-48 hours, of infection with active virus: Slight cellular infiltration chiefly with polymorphonuclear cells in meningeal and perivascular areas, and mild disorganization of lining cells accompanied by similar cellular infiltration of the ependyma and chorioid plexuses. Whether the changes are induced by cortical hemorrhages—almost invariably concomitants of the inoculation—or by the inoculum itself is not known. The degree of this reaction therefore differentiates it from that caused by virus. Not all viruses respond in this way, for example, our finding for herpes,[39] later confirmed by others for poliomyelitis virus; herpes virus can multiply in guinea pigs' brains without evoking any distinctive signs of disease or histopathological lesions.

amount of equine virus is given it will gain a temporary foothold in the vaccinated, apparently highly immunized, animals even causing certain changes which may be reflected as an abortive, or possibly an inapparent infection.

Nonspecific Resistance.* Interference Phenomenon

Since the living cell is needed for the multiplication of viruses, its condition is necessarily linked with the fate of the latter. One type of nonspecific resistance may result from an alteration of the normal cell by (1) specific virus action or (2) general disturbance of metabolism as in malnutrition. Another type of nonspecific resistance may be due to (3) mechanical factors influencing the progression of a virus from its portal of entry.

1. *Interference due to virus action on cells:* When a tissue harboring a virus is exposed within a definite time to another virus, or when two viruses are injected at the same time, and one inhibits the multiplication or modifies the action of the other, the process involved, which is not dependent on neutralizing antibody or on serological specificity, is designated the interference phenomenon. The interest of this laboratory in the problem concerned additional examples of this reaction, particularly in the CNS tissues; the classification of viruses on the basis of their "interfering" capacity and studies of the principles involved in production of the resistance.

Rabbits or guinea pigs convalescent from experimental Eastern or Western equine virus infection induced by intracerebral inoculation withstand, during a period of two weeks, similar injection of both viruses, also of that of vesicular stomatitis.[37,38] This resistance is not brought about by introduction into the brain of subinfective amounts of active or formalin-inactivated virus, nor does it depend on neutralizing antibody in the circulation or brain. The resistance is confined to the CNS because heterologous virus after peripheral inoculation is not prevented from circulating in the blood stream. One virus given in the brain interferes therefore with another given in the brain. Moreover a small dose of Theiler's virus (mouse poliomyelitis[40]) introduced intracerebrally into mice and giving rise to incubation periods of 6 to 14 days induces from the fourth to the tenth day, an increasing resistance

* To be noted is that reference is made to "immunity" when specific antibody or the general principles of specificity are involved; to "resistance" as now used in this section when nonspecific factors are dealt with.

to large doses of Western equine virus given intracerebrally. Not only does the Theiler virus interfere in mice with Western virus but also with Eastern and certain other neurotropic viruses—the tests are still being carried on.[37,38] From what has been said, one should hesitate to use animals recently tested, as is done in laboratory identification of newly isolated viruses or strains for fear of encountering this nonspecific phenomenon.

2. *Interference due to general disturbance of metabolism:* It was first shown experimentally by Rous (1911) for the Rous sarcoma and in this laboratory (1925) for a virus such as foot-and-mouth disease[41] that thin, marasmic, or ill-nourished animals are resistant to inoculation;* moreover, the resistance is nonspecific—it is not evoked through the action of neutralizing antibody.[41] Rivers and colleagues have also demonstrated that rabbit skin irradiated with ultraviolet light is less susceptible to vaccine virus than untreated skin.

Nasal chemoprophylaxis: Another form of nonspecific resistance studied here is that following nasal instillation of certain chemicals (tannic acid; alum; zinc sulphate as used by Schultz, etc.) to prevent viruses which progress along the olfactory pathway from reaching the CNS.

The chemoprophylaxis, as described, was first observed in 1934 in experimental equine encephalomyelitis;[42] it was promptly applied to various viruses including experimental poliomyelitis;[43,44] in the latter case it was 100 per cent effective. When, however, it was applied to human beings during an epidemic of the malady, the method failed for the reason, as now believed, that the virus is in nature not ordinarily transmitted via the olfactory pathway.

The mechanism underlying the action of nasal chemoprophylaxis is still being studied, although it was thought that the chemicals sprayed or instilled into the nasal cavity damaged in some way the olfactory mucosa (neurons), thus preventing infection by the virus; restored susceptibility followed the restitution of the membrane (Schultz and Gebhardt).

3. *Interference due to mechanical factors:* Nonspecific resistance is not always due to cellular changes but can arise from mechanical factors such as blocking the progression of virus from its portal (King; Sprunt and colleagues). In this laboratory[45] it was shown that following the use of hypertonic solutions producing edema, a virus such as

* Cf. the dictum of Rivers: "Perfect health is no protection against poliomyelitis!"

herpes febrilis progressed more actively with an enhancement of obvious signs of infection. On the other hand, substances inducing acute inflammation and partial or total blockade of the lymphatic system, retarded or even inhibited virus infection.

In summarizing the theoretical aspects of the problem of interference, especially that due to virus action which is designated in this laboratory as "an acquired nonspecific cellular resistance,"[37] one should draw attention to the susceptibility of normal cells and the resistance of "abnormal" ones to virus. Just what the mechanism of resistance is is still unknown. One view is that virus affects the receptor or receptor substance of susceptible cells thus inactivating it (after Hirst who postulates this to be the mechanism of hemagglutination by influenza virus). If any one virus interferes with any other, the phenomenon then is completely nonspecific—cells made "abnormal" through virus action become refractory to infection by another. If, on the other hand, only specific cell receptors are inactivated, without otherwise affecting the normality of the susceptible cell, it may then maintain the "interfering" power of virus. In such an event one can conjecture on the possibility of prophylaxis, after the manner of chemoprophylaxis; further study may show the way.

At this moment the insusceptibility of a host to infection depends on a number of factors, perhaps more than one acting concomitantly. In experimental equine encephalomyelitis, certain of these factors are, first, the *systemic strain-specific immunity* in which the degree is correlated with the titer of neutralizing antibody, and can be active, as: (a) in recovery from disease or from inapparent infection; (b) after vaccination, or (c) in the anamnestic state consequent to recovery, vaccination, or exposure to active virus) or passive (as from maternal transfer of antibody or from injection of antiserum). The second factor is *cellular resistance* independent of neutralizing or other specific serological antibody and is nonspecific, developed through virus action or through cellular changes (nutritional, endocrinological, genetic) not induced by a virus. Another form of nonspecific resistance is due to *mechanical factors preventing progression of a virus* from its portal. Still another is *maturation*—the increasing insusceptibility with age which is correlated with increasing capacity to produce virus antibody, or with cellular changes ("barriers") or with both. This is an instance in which time has not served to simplify nature!

One word before closing this lecture. The whole story is not told, yet the speakers must save themselves from the quicksands of illusion; they must give no other impression than that their colleagues and themselves are at the very threshold of knowledge of the subjects here discussed.

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DIFFERENCES IN THE NATURE OF ANTIBACTERIAL ACTION OF THE SULFONAMIDES AND PENICILLIN AND THEIR RELATION TO THERAPY*

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THE therapeutic effectiveness of the sulfonamide drugs is due solely, insofar as can be determined, to their bacteriostatic property—the ability to restrain the growth of the organisms. They do not exert a primary killing effect. The killing of the infectious agent is left to the normal body mechanisms, apparently chiefly through phagocytosis which occurs most actively when specific antibody is present, whether because of active immunization of the host by the infectious agent during the course of the disease itself or else supplied artificially through the use of immune sera—a procedure which many consider to be seldom necessary nowadays except in the case of bacteria which produce exotoxins, such as the gas gangrene anaerobes. Dependence on specific immunity is thus an important limitation to the effectiveness of the sulfonamides, since, unless phagocytosis is active the infectious agent may not be eradicated. A more ideal chemotherapeutic drug is one which will kill the microörganisms without such complete dependence on the complex antibody-complement-phagocyte system. There are indications that in the animal and human body penicillin exerts this effect to a considerable degree and its greater usefulness in many cases may be due to its killing or bactericidal property.

It seems proper to review certain parts of the evidence on which the foregoing observations with respect to sulfonamides and penicillin are based. In the case of the sulfonamides this can be very clearly dem-

* A portion of the material in this paper was presented October 9, 1944 at the 17th Graduate Fortnight of The New York Academy of Medicine under the title, "Factors which influence the choice of antibacterial agents."

This study was supported in part by the Commission on Pneumonia, Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army.

Part of the penicillin used in this study was supplied by Merck and Company through the courtesy of Dr. D. F. Robertson, and part through the Committee on Medical Research of the Office of Scientific Research and Development.

onstrated by a consideration of the therapy of experimental pneumococcal infections of mice.[1]

If mice are infected with pneumococcus Type I and treated with a sulfonamide such as sulfapyridine or sulfadiazine, therapy must be continued for a period of 48 to 72 hours for recovery to take place. The same amount of drug given on a single day is ineffective. Experimentally, in other groups of mice, it can be shown that a period of 60 to 72 hours is required for the development of active immunity. Pneumococcus Type I is a good antigen so that between 90 and 100 per cent of mice become immune during this period. The sulfonamides are very highly effective against infections with Type I and uniformly, between 90 and 100 per cent of infected mice recover following treatment for three days. In other words, the proportion of animals recovering following sulfonamide therapy corresponds closely to the proportion of mice which becomes immunized by a single injection of killed Type I pneumococci.

In the case of pneumococcus Type III the story is similar but at the other end of the scale. Although most strains are as susceptible as Type I to the bacteriostatic action of the sulfonamides *in vitro*, in experimental infections of mice these compounds are relatively ineffective. Between 5 and 10 per cent only of mice infected with Type III pneumococcus recover following sulfonamide therapy continued for as long as five days. During the period of drug administration the animals remain well, but when the drug is stopped most of the mice die. In other words, the drug has exerted a bacteriostatic effect, but due to some failure on the part of the host, destruction of the bacteria has not occurred. There is evidence that this failure is due to the inability of the host to produce antibodies to pneumococcus Type III, except in a small proportion of cases. Pneumococcus Type III, relatively speaking, is a poor antigen. If groups of mice are injected with a heat-killed vaccine of this organism, only 5 to 15 per cent develop active immunity against even a very small dose of living organisms. The proportion of animals which survive following sulfonamide therapy is about the same as that which becomes immunized to pneumococcus Type III when injected with a heat-killed vaccine.

Admittedly this is not hard and fast evidence that in strains of organisms of equal virulence and *in vitro* susceptibility, the difference in effectiveness of the sulfonamides in curing infections with these bac-

TABLE I

RELATION OF THE EFFICACY OF SULFONAMIDES TO ANTIGENIC RESPONSE OF MICE TO PNEUMOCOCCI

Pneumococcal Type	*% surviving following sulfonamide therapy*	*% immunized by heat-killed vaccine*
Type I	90-100	90-100
Type II	50-60	50-60
Type III	irregular 0-10	5-10

Virulence of all strains for mice: 10^{-8} cc of culture.
Susceptibility of strains to bacteriostatic action of sulfonamides *in vitro* the same within the limits of error of test.

teria is due to differences in the immune response of animals to them. Further evidence that the immune response is of primary importance may be got from a study of infections with Type II pneumococcus. In its response to the sulfonamides in experimental infections, Type II lies midway between Type I and Type III. The sulfonamides will effect cure of approximately half of the mice infected with Type II. Correspondingly, if one injects mice with a single dose of a heat-killed vaccine of Type II pneumococcus, it is found that only about half of the animals develop immunity. The evidence would appear to be quite clear therefore, that in experimental infections of mice with different strains of pneumococci of the same virulence and *in vitro* susceptibility to sulfonamides, but differing in their antigenic potency, the therapeutic effect of the drugs is directly proportional to their antigenicity. A summary of these observations is given in Table I. From these data it can be seen that the effectiveness of the drugs parallels the antigenicity of the bacteria to a remarkable degree.

The antigenicity of these three types of pneumococci in other animals including man, parallels roughly that observed in mice, with Type I an excellent antigen and Type III relatively poor. It is also of considerable interest that in man, infections with Type III pneumococcus respond to sulfonamide therapy less well than infection due to other types. The overall mortality in pneumococcal pneumonia treated with sulfonamides is in the neighborhood of 10 per cent, whereas with

Type III the mortality is between 20 and 25 per cent.

Failure of action of sulfonamides in localized infections: For a time it was thought that sulfonamide inhibitors were solely responsible for the failure of sulfonamide therapy in certain localized infections such as empyema. Extensive study of experimental pneumococcal empyema in rabbits[2] indicates, however, that this is not uniformly so, since in some instances relatively enormous doses of sulfadiazine injected locally failed to sterilize the cavity even though we were unable to detect the presence of any sulfonamide inhibitor whatsoever, and the reaction of the pleural exudate was neutral or slightly alkaline. Factors other than these must then be sought in order to explain the lack of effect. It should be noted that the organisms used to produce the empyema remained highly susceptible to the bacteriostatic action of the drugs *in vitro*. The reasons for failure of the sulfonamides locally may be that antibody in sufficient quantity does not enter the lesion and that most of the leukocytes in the exudate are dead or damaged and no longer actively phagocytic. Absence of antibodies and functional phagocytes has seemed to us to be the most probable explanation, since, although a bacteriostatic concentration of sulfadiazine could be achieved in the absence of demonstrable inhibitors, the organisms were not destroyed. This is further evidence for the importance of the normal host defense mechanisms in the action of the sulfonamides. It is apparent that the purely bacteriostatic action of the sulfonamides and lack of a bactericidal effect place serious limitations on their usefulness.

Bactericidal action of penicillin: Penicillin has changed entirely our approach to the therapy of local infections such as empyema. Not only does it sterilize a pneumococcal empyema cavity, but this effect may occur within 4 to 8 hours. This is true both in experimental animals and in man. It seems most likely that the dramatic response to penicillin injected locally under such circumstances is due to the direct bactericidal action on the organisms as opposed to the bacteriostatic effect of the sulfonamides, which requires to be supplemented by antibody and phagocytes.

Hobby, Meyer and Chafee[3] demonstrated that penicillin exerts a bactericidal effect *in vitro* and this observation has been confirmed repeatedly by others. Killing does not occur instantaneously as when a culture of bacteria is dipped into boiling water, nonetheless the effect is quite definite. It should be noted, however, that usually not all of the

organisms are killed *in vitro*, a few remaining alive.

When penicillin is injected locally into empyema cavities, the bactericidal effect is even more striking than that which occurs *in vitro*, provided one is dealing with penicillin-sensitive organisms. What evidence is there for a bactericidal effect in infections other than empyema?

In patients treated for anterior urethritis by intramuscular or intravenous injection of penicillin, it has been noted that viable gonococci may disappear within a few hours. Similarly, spirochetes may disappear from a primary chancre within a matter of a few hours following initiation of treatment. Both of these observations indicate that penicillin is exerting a direct killing effect on the infecting microörganisms. On the other hand, in the case of anterior urethritis treated successfully with a sulfonamide, similar dramatic disappearance of the gonococci does not occur, but may take several days. It may be argued, however, that this does not rule out specific immunity in the case of penicillin therapy, since by the time treatment is begun the microörganisms have been present in the body for a sufficient period to provoke a specific immune response. This would be true even if chemotherapy is begun on the first day of clinical disease since the organisms have already been present during the incubation period which might provide sufficient time for the production of immune bodies, although the amount might be very small and not detectable by the methods available. In this regard it has been shown in rabbits that active immunity in the case of pneumococci appears before specific antibodies are demonstrable in the circulating blood.[4]

The lesser role played by specific antibody in penicillin-treated infections is demonstrated by experiments in mice infected with pneumococci and treated with penicillin.

If mice are infected with pneumococcus Type I and treated with penicillin in three divided doses at 4 hour intervals thereafter on the day of infection only, a considerable proportion of the animals survive. Under this scheme the last dose of penicillin is given 8 hours after the animals were infected. The recovery rate depends in good part upon the amount of penicillin injected and reaches a figure of approximately 75 per cent when large amounts are given. It seems unlikely that an effective concentration of drug is present in the animal body for more than 12-16 hours after the infection was initiated, yet during this period penicillin has been able to eradicate the infection completely from most

TABLE II

EFFECT OF PENICILLIN GIVEN ON A SINGLE DAY TO MICE INFECTED WITH PNEUMOCOCCI

Pneumococcal Type	*Virulence for mice*	*Bacteriostatic dose in vitro units per cc*	*Per cent of Mice Surviving*			
			Oxford Units per Day			
			300 & 600	75	30	15
Type I	10^{-8} cc	0.05	74.1	57.8	23.5	16.7
Type II	10^{-8} cc	0.025	72.5	15.0	8.3	—
Type III	10^{-8} cc	0.05	15.4	—	0.0	—

Infecting inoculum 10^{-2} cc of culture.
Penicillin given in 3 divided doses at 4 hour intervals.
The rate of killing by penicillin (bactericidal effect) *in vitro* was the same for each of the three types.

of the mice. Explanation of this phenomenon is difficult unless penicillin exerts a direct bactericidal effect in the presence of a systemic infection with pneumococcus Type I. The participation of specific antibodies, in the light of our present knowledge, seems unlikely, although not completely ruled out. These data are shown in Table II.

When either 300 or 600 Oxford units of penicillin are given over an 8 hour period, 74 per cent of the Type I infected animals survive—the maximum effect being achieved at 300 units, with no improvement on increasing to 600 as shown in Table II. At a dosage of 75 units about 58 per cent survive; this decreases to 23.5 per cent at 30 units and is only 16.7 per cent when 15 units are used. It should be noted that 15 units per day in 3 divided doses of 5 units each, given on each of 4 successive days will effect recovery in almost 100 per cent of the animals. However, with this dosage given on a single day, the recovery rate is low.

As previously stated, sulfadiazine administered for a single day is ineffective, even though given at maximum dosage and using the same strain of pneumococcus Type I as in the penicillin experiments. There is apparently a considerable difference in the nature of the antibacterial action of the drugs, sulfadiazine being purely bacteriostatic, whereas penicillin is rapidly bactericidal particularly at high dosage. It has been

estimated that against organisms susceptible to both, penicillin is about 1000 times as active as sulfadiazine. This purely quantitative difference is most important, but it seems proper to emphasize that the qualitative difference may be of even more significance. At lower dosage penicillin behaves more like sulfadiazine, that is, as a bacteriostatic agent, but in a high dosage it is bactericidal.

Pneumococcus Type II behaves somewhat similarly to Type I in mouse infections treated with penicillin, however, it is only at the larger dosages that treatment for a single day has much effect, as shown in Table II. With Type III the effect is considerably less, so that even at dosage of 300 or 600 units per day only 15 per cent of the mice survive. It should be noted that with all three types the virulence was the same in that 10^{-8} cc of culture (1-5 organisms) killed control mice uniformly. The infecting inoculum in each case was 10^{-2} cc of culture corresponding to 1,000,000 lethal doses. *In vitro* the amount of penicillin required to produce bacteriostasis was the same within the limits of error of the dilution method of assay, and each of the strains was killed at the same rate by penicillin *in vitro*. From these studies it is apparent that one cannot carry over to the animal body the results of studies of susceptibility to penicillin *in vitro*, since all three types of pneumococcus behave very similarly *in vitro*, yet *in vivo* differ greatly.

The differences in behavior *in vivo* remain unexplained. It may be suggested that part of the bactericidal effect observed *in vivo* with penicillin is due to the participation of some component of the animal body. This component is most effective against Type I in the presence of penicillin, less so against Type II and even less effective against Type III. Despite these differences in respect to the three pneumococcal types it is apparent that penicillin exerts a direct bactericidal effect when large doses are used in the treatment of mice infected with pneumococci of these three types.

If therapy with penicillin is prolonged for four days, a high proportion of cures can be obtained even in the case of infection with pneumococcus Type III, as shown in Table III. The rate of recovery is proportional to the dosage of penicillin, and for both Type II and Type III much larger amounts are required than for Type I, which, it should be recalled, responds best of the three types to treatment given on one day only (Table II). The degree of response to the four day treatment at all dosage levels, but particularly 75 units per day, cor-

TABLE III

EFFECT OF PENICILLIN GIVEN FOR 4 DAYS TO MICE INFECTED WITH PNEUMOCOCCI

Pneumococcal Type	*Virulence for Mice*	*Bacteriostatic in vitro units per cc*	*Per cent of Mice Surviving*			
			Oxford Units per Day			
			300	75	30	15
Type I	10^{-8} cc	0.05	—	100.0	97.4	100.0
Type II	10^{-8} cc	0.025	94.7	60.0	8.3	—
Type III	10^{-8} cc	0.05	88.5	46.2	3.9	—

Infecting inoculum: 10^{-3} cc of culture.
Daily dose of penicillin was administered in 3 divided doses.
The rate of killing by penicillin (bactericidal effect) *in vitro* was the same for each of the three types.

responds roughly to what we know of the antigenicity of these three types. That the development of specific immunity has probably only a minor bearing on recovery, however, is shown by the data in Table IV.

Mice recovering from infection following treatment with penicillin were tested ten days later for specific active immunity by reinfecting them with relatively small doses of homologous strains of pneumococci, as shown in Table IV. The reinfecting dose was very small for all three types, but especially for Type II and Type III. It was found that most of the mice recovering from infection with Type I were immune to reinfection. Approximately one-half of the type II infected mice only had developed immunity even though they had previously recovered from infection with the homologous strain. Of the type III infected mice which had recovered following penicillin treatment, only 12 per cent were immune to reinfection with as little as 100 lethal doses. The percentage of animals immune to the respective types in this penicillin treated series is almost exactly the same as had previously been found for normal mice immunized with a single dose of heat-killed organisms as is shown in Table I. In other words, penicillin did not affect the immune response in demonstrable fashion, and most of the type III infected mice and half of the type II mice recovered without evidence

TABLE IV

ACTIVE IMMUNITY IN PENICILLIN TREATED MICE

Type of Pneumococcus	*Reinfecting Dose (M.L.D.)*	*No. of Mice*	*Per cent Immune (Survivors)*
Type I	1000	38	94.2
Type II	100	62	51.6
Type III	100	33	12.1

Mice had recovered from original infection following treatment with penicillin. Immunity to reinfection with homologous strain was tested 10 days after original infection.

of the development of specific immunity.

From the data presented in Tables II, III and IV it appears probable that an immune response to the infecting agent is not as important a prerequisite for successful therapy with penicillin, as seems to be true for the sulfonamides. With penicillin therapy on a single day only, approximately ¾ of type I and II infected mice recover, presumably before specific immunity has had a chance to develop. Similarly, almost 90 per cent of type III infected mice recovered following high dosage of penicillin for 4 days, and on subsequent test only 12 per cent of the recovered mice were immune to type III. An antibody response, therefore, seems of much less importance to successful therapy with penicillin than with the sulfonamides.

The possibility still remains that an effective level of antibodies might develop within 12-16 hours of infection in the case of type I and type II, and that the amount developed in animals recovered from type III infections is too small to detect even by the most sensitive of methods: that is, testing for the presence of active immunity. This is unlikely. The tentative conclusion has been drawn that though specific antibody is probably not the somatic component participating in the bactericidal effect of penicillin in systemic infections, some auxiliary component or components, whose nature is at present unknown, do participate.

It has been assumed in the foregoing discussion that certain more obvious factors have been taken into consideration in the choice of a

therapeutic agent. Among the most important of these are that the microörganisms causing the infection must be susceptible to the action of the drug employed; that the drug can diffuse into or be introduced directly into the infected area, and be maintained there in adequate concentration; and that local factors such as inhibitors or unfavorable pH do not nullify its action.

Drug fastness or resistance: Sulfonamide fastness may be an inherent property of all members of a species or genus of bacteria. For example, indifferent streptococci have been found to be highly resistant to this group of drugs even though they have not previously been in contact with a sulfonamide. One may speak of such fastness as natural in contrast to the acquired fastness which may develop in a previously susceptible strain of bacteria during the course of treatment, particularly if the latter be prolonged.

Acquired fastness is a commonplace finding during sulfonamide therapy, particularly with staphylococci, gonococci and to a lesser extent, pneumococci. Staphylococci possess the ability to become fast fairly rapidly not only to the sulfonamides, but also to gramicidin and penicillin. For this reason, in addition to the circumscribed, walled-off lesion which they cause characteristically, staphylococcal infections present a somewhat unique problem in chemotherapy.

In the first few years following the introduction of the sulfonamides, the proportion of cures in acute anterior urethritis was variously reported as between 85 and 95 per cent. Since then, however, the cure rate has fallen considerably under sulfonamide therapy. It is held by some that the decrease in cures is apparent rather than real and reflects principally improvement in the methods used to determine whether cure has occurred. On the other hand it has been demonstrated that sulfonamide-fast gonococci are encountered commonly at present, whereas during the early days of sulfonamide therapy this is believed not to have been the case. In many clinics, determination of the sulfonamide resistance of gonococci is used routinely as a guide to therapy. This would seem to be an important part of diagnosis, since susceptibility of the infecting strain as measured *in vitro* appears frequently to parallel the susceptibility of the human infection to sulfonamide therapy.

If a strain of bacteria is fast to one sulfonamide, for example, sulfadiazine, it is fast also to other sulfonamides such as sulfapyridine or sulfathiazole. Moreover, fastness once acquired persists in organisms

through an indefinite number of transfers in artificial culture media or animal passages. Acquired fastness to the sulfonamides, however, does not extend to penicillin, which may be used for the treatment of sulfonamide-fast infections.

The evidence obtained up to the present time indicates that penicillin fastness develops less readily than fastness to sulfonamides. In addition to this, penicillin causes the organism to disappear more rapidly, so that its use appears to be distinctly advantageous in infections where fastness to sulfonamides develops frequently, such as in gonorrhea.

The possibility of sulfonamide fastness should be kept in mind in other infections which respond poorly to therapy. It is desirable, if possible, to determine whether the infecting organisms are fast when tested *in vitro*, since it is only by such measurements that one can obtain eventually an appreciation of the problem of fastness and whether or not it is increasing progressively.

Inhibitors of chemotherapeutic drugs: Some sulfonamide-susceptible strains of bacteria produce inhibitor normally during growth, but in amount insufficient to interfere with the bacteriostatic action of the drug. On the acquisition of sulfonamide fastness the amount of inhibitor may be increased greatly with some strains, but fastness of equal degree may be present without any measurable increase in inhibitor production. Sulfonamide inhibitors may also appear in certain lesions, particularly if tissue breakdown is occurring, and in some instances account for failure of sulfonamide therapy. Failure of sulfonamide therapy in localized purulent infections, however, may occur in the absence of inhibitors which are demonstrable by the usual *in vitro* techniques, as mentioned previously in the case of experimental empyema of rabbits.[2] In some instances no inhibitor could be demonstrated in the pus, yet local therapy with sulfonamides was completely ineffective in sterilizing the lesions. In addition, Tillett[5] has shown that empyema fluid from patients does not always contain sulfonamide inhibitor, yet local therapy with these drugs is unsuccessful. As suggested earlier, the failure of local sulfonamide therapy may be based commonly on factors other than inhibitors, and the success of penicillin under these circumstances due not so much to the absence of penicillin inhibitors as to the bactericidal properties of this compound.

In passing, it may be mentioned that certain organisms, both gram positive and negative, may elaborate an enzyme, penicillinase, which

destroys penicillin. The importance of this enzyme as a cause of failure of penicillin therapy has not been adequately assessed.

Nature and location of the lesion: Lesions produced by staphylococci characteristically localize early and become surrounded by a pyogenic membrane which prevents diffusion of chemotherapeutic agents into them. If therapy has not been begun before walling-off has taken place, it may be necessary to eradicate the local lesions by surgical procedures or else introduce adequate concentrations of penicillin directly. The sulfonamides exert little effect on localized staphylococcal infections, though they may be lifesaving in preventing overwhelming systemic infection. Their failure locally may be attributable to the presence of sulfonamide inhibitors, acid reaction of the pus or absence of specific antibodies and functional phagocytes which appear to be essential for the therapeutic action of these bacteriostatic compounds. It seems probable that the failure of the sulfonamides in the treatment of tuberculous infections may be attributable to the same factors, since it has been shown *in vitro* that the tubercle bacillus is susceptible to their bacteriostatic effect.

Factors other than a pyogenic membrane may prevent diffusion of a chemotherapeutic agent into the involved area. Most of the sulfonamide drugs pass readily into the cerebrospinal fluid and hence treatment of meningitis with sulfonamides can be carried out without recourse to intrathecal administration. This is a distinct advantage in the therapy of meningococcal meningitis. Penicillin is extremely active against the meningococcus but following intramuscular or intravenous injection very little of the compound appears in the cerebrospinal fluid. It must therefore be injected intrathecally, as well as by other routes in treating meningitis.

Difference in the rate of antibacterial action of the sulfonamides and penicillin: An additional factor which should be taken into consideration in choosing a chemotherapeutic agent is the difference in the rate of antibacterial action of the sulfonamides and penicillin. It has been demonstrated both *in vitro* and in the animal body that a lag phase occurs in the action of the sulfonamides. For a period up to four hours or even longer, susceptible bacteria exposed to an effective concentration of a sulfonamide, multiply at a normal rate. Following this initial lag phase, multiplication ceases. Under many circumstances this delay in bacteriostasis may not be important. On the other hand, in a rapidly

progressing infection such as fulminating meningococcal meningitis, or in an infection not treated until considerably advanced, the lag phase in sulfonamide action might conceivably be sufficient to permit a fatal outcome. Similarly, in infections caused by bacteria which produce potent exotoxins such as the gas gangrene anaerobes, the amount of toxin produced by the organisms in a massive area of infection during the sulfonamide lag phase, might also be seriously detrimental. This lag phase, so characteristic of the antibacterial action of the sulfonamides, is not encountered with penicillin *in vitro*, and so far as present data go, does not occur to a significant degree in the animal body. Under the conditions enumerated immediately above, penicillin would appear, therefore, to be a more appropriate therapeutic agent. This view would appear to be even more valid, if one adds to the lack of a lag phase the primary bactericidal properties of this drug as discussed earlier, which require less active participation of the body defense mechanisms than in the case of the sulfonamides.

Summary

1. The sulfonamide drugs are strictly bacteriostatic and require for their most effective action the participation of specific antibody and an active phagocytic system in curing infections due to susceptible organisms.

2. Penicillin exerts a direct bactericidal effect in the animal body as well as in the test tube, and hence its therapeutic efficacy is not as dependent on the development of specific immunity as in the case of the sulfonamides. This does not imply that specific immunity may not be very desirable as an adjunct to penicillin therapy under certain circumstances.

3. Equal and marked susceptibility of bacteria to the effect of penicillin in the test tube, even of members of the same species, as in the case of pneumococcus Types I, II and III, does not mean that infections caused by them will respond equally well. It should be noted that the same is true of sulfonamide therapy, since bacteria of the same susceptibility *in vitro*, for example the above strains of pneumococcus, Types I, II and III, differ greatly in their response to the sulfonamides in the animal body.

4. Failure of sulfonamide therapy in localized purulent infections, even though introduced directly into them, is due in all likelihood to a

number of factors: the presence of sulfonamide inhibitors, an unfavorable pH, development of sulfonamide fastness, and the absence from the lesions of specific antibody and functional phagocytes.

5. Under certain circumstances the immediate antibacterial effect of penicillin, as contrasted with the lag phase characteristic of the action of the sulfonamides, may be of importance in determining which therapeutic agent should be used.

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